

A chronic ulcerative colitis model in rats

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Subject headings colitis, ulcerative; disease model, animal; rats

INTRODUCTION

In recent years, there have been many reports about animal model to investigate drugs for inflammatory bowel diseases (IBD). The experimental animal model often used is acetic acid-induced damage of colonic mucosa. In the present study, this animal model was investigated by administering various concentrations of TNBS.

MATERIALS AND METHODS

Materials

Animals Wistar male and female rats weighing 258 ± 25 g were used in this study. They were provided by the Department of Experimental Animals of Beijing Medical University. They were housed in rack-mounted cages with a maximum of 6 rats, and were fasted for 12h with access to water ad libitum before experiment.

Reagent Fifty mmol/L (pH=6.0) phosphate buffer, 0.5 % hexadecyltrimethylammonium bromide (HTAB, Beijing Xizhong Chemical Plant) in 50mmol/L (pH=6.0) phosphate buffer, 50mmol/L (pH=6.0) phosphate buffer containing 16.7% (g/L) o-dianisidine Dihydrochloride (Sigma chemical Co.) and 0.0005% hydrogen peroxide, TNBS (Sigma chemical Co. 5% w/v solution) solution of 30% ethanol, 20% (w/v) ethyl carbamate in 0.9% saline.

Instrument T25 Ultra-tukrax (German, JANKE & KUNKEL IKA-Labortechnik). 4710 series

Ultrasonic homogenizer (Cole-Parmer Instrument Co. America), GL20A Refrigerated Centrifuge (Hunan Instrument and Meter Plant China), UV-260 Spectrophotometer (Shimuduzu Co. Japan), PHSJ-4 pH meter (Shanghai Leici Instrument Plant China), Libror EB-2080M Electronic Animal Balance (Shimuduzu Co. Japan).

Methods

Effect of various TNBS doses on myeloperoxidase (MPO) activity, colon damage and weight A total of 30 rats were randomized into five groups, 6 rats each group (in a cage), consisting of a 30% ethanol control group as well as four dose TNBS groups. The animals were anesthetized with 20% ethyl carbamate (ip, 6mL/kg), and 0.5mL of either 30% ethanol (controls) or various concentrations of TNBS was slowly administered into the lumen of the colon via the anus using a rubber catheter (12cm long, external diameter 2mm). The rats were killed after 3wk, and the distal colon (8cm) was removed, opened longitudinally and washed to remove lumina contents, colon wet weight was weighed, and colonic injuries were evaluated. The excised colon was pinned out on a wax block washed with 0.9% saline and assigned a code number. The colon was immediately examined under a stereomicroscope and any visible damage was scored on a 0-5 scale (Table 1). Small sections of colon were taken from two distinct areas from each colon and placed in 10% formalin for histological examination. The colon was fixed, cut longitudinally into 5mm sections, stained with hematoxylin and eosin. The second segment (200mg-400mg) was immediately frozen for subsequent estimation of MPO activity^[1].

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Table 1 Criteria for scoring the gross morphologic damage

Score	Gross morphology
0	No damage
1	Localized hyperemia with no ulcers.
2	Liner ulcers with no significant inflammation.
3	Liner ulcers with inflammation at one site.
4	More sites of ulcers and inflammation, the size of ulcers <1cm.
5	Multiple inflammations and ulcers, the size of ulcers ≥1cm.

The relationship of MPO and time-course Based on the results of dose-response studies, the dose of

TNBS used in subsequent experiments was 100 mg/kg per rat. A total of 40 rats (5 rats per cage) administered a single intracolonic dose of TNBS ethanol solution (0.5 mL/rat). In control experiments, 5 rats received 0.5 mL 30% ethanol. At various times (24 h and 1 wk-8 wk) after intracolonic administration of TNBS or one of the control solutions, 5 rats from each treatment group were randomly selected and killed, the colon tissue MPO activity was determined as the indices of inflammation.

Statistical analysis The data were expressed as $\bar{x} \pm s$, and analyzed using the Student's *t* test to compare the difference.

RESULTS

Assessment of MPO activity, colonic weight and damage score

The severity of colonic damage induced by TNBS increased with the dose (Figure 1). Rats that received the lowest dose of TNBS (25 mg/kg) had damage scores, colon weights and tissue levels of MPO activity were not significantly different from the control animals treated only with 30% ethanol vehicle ($P > 0.05$). When doses of TNBS (50 mg/kg - 150 mg/kg) were used, the damage scores, colon weights and tissue levels of MPO activity increased in a dose-related manner and there was a significant difference compared with control (30% ethanol).

Histological examination

Three weeks after TNBS/ethanol administration, the bowel wall was basically normal in the 25 mg/kg group, and "string of beads" was found in 1 rat. Medium hemorrhage, edema and ulcers, cryptoabscess in the mucosa were observed in animals that received 50 mg/kg of TNBS, in TNBS group (100 mg/kg), the bowel lumen became narrow with thickened wall (2 mm-3 mm), on the bowel lumen mucosal surface area there was adherent membrane with brown black, liner ulcers (1 mm-6 mm), proliferous lymphocyte tissue, inflammatory granulomas and submucosal neutrophils infiltration. Macrophages, lymphocytes, fibroblasts, and cryptoabscess were also observed. The TNBS (150 mg/kg) group had noticeable ulcers and inflammatory granulomas in their colon, neutrophil infiltration was obviously observed in mucosa and sub mucosa extensive necrosis of the colonic mucosa and exfoliation of the epithelia were found in other rats with intact

muscularis. In cases of severe ulcers, the colon had often adhered to surrounding intestinal tissues and abdominal wall (Figure 2).

Time-course study

A single instillation of TNBS at the dose of 100 mg/kg into the rat colon produced chronic ulcers and inflammation which had persisted for up to 7 wk. MPO activity reached a maximum value at 3 wk after TNBS, and was followed by a gradual reduction in activity. At 3 wk the MPO value was at near baseline level (Figure 3).

DISCUSSION

TNBS is a hapten, when it is bound with a substance of high molecular tissue proteins, it will turn into an antigen. It has been shown that it can elicit immunologic responses, induce generation of colitis^[2,3]. The histological features of the animals received TNBS (50 mg/kg-150 mg/kg) were chronic inflammation, relatively long duration of inflammation and changes in various inflammatory mediators such as prostaglandin E_2 , thromboxane B_2 , leukotriene B_4 , 6-keto-prostaglandin $F_{1\alpha}$, leukotriene C_4 , platelet activating factor and interleukin. This model is characterized by the simple process and reproducible colonic damage, inexpensive and short duration of the experiment, long-lasting damage with inflammatory cell infiltration and ulcers. Thus, the model is rather suitable for the assessment of the effects of potential agents. In the present study, the dose of TNBS producing a moderate colonic inflammation and ulcers was about 100 mg/kg, the severity of colonic inflammation induced by TNBS increased with the dose administered. So a TNBS dose of 100 mg/kg was chosen for an appropriate experimental dose, the results were similar to the reports in the literature^[4,5].

There was extensive colonic mucosal and submucosal damage characterized by infiltration of inflammatory cells and ulcers after different doses of TNBS (50 mg/kg - 150 mg/kg) were administered into the colons of rats. After the animals received TNBS (100 mg/kg), in acute phase, extensive infiltration of inflammatory cells constituted the main part; in chronic phase, the inflammatory granulomas and ulcers induced by TNBS made up the main part. MPO is an enzyme found in the neutrophils, and can be used as a quantitative index of inflammation in colonic tissue^[1]. MPO activity may be regarded as an index of inflammation damage^[6].

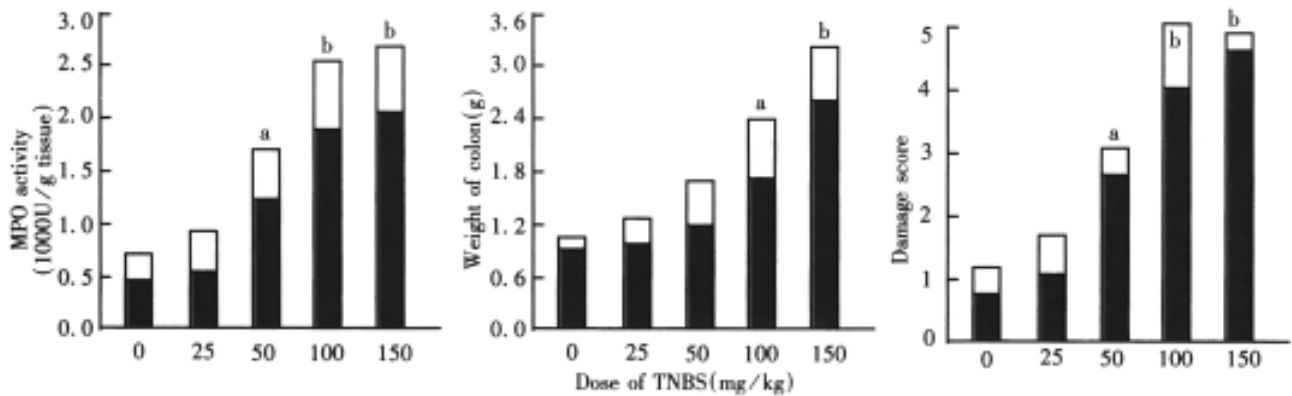


Figure 1 Rats were killed 3wk after intracolonic administration of 25 mg/kg-150 mg/kg of TNBS. Colonic damage, colon weight and tissue MPO activity were assayed. Data of TNBS-treated rats were compared with control (30% ethanol) group by Student's *t* test, ^a*P*<0.05, ^b*P*<0.01.

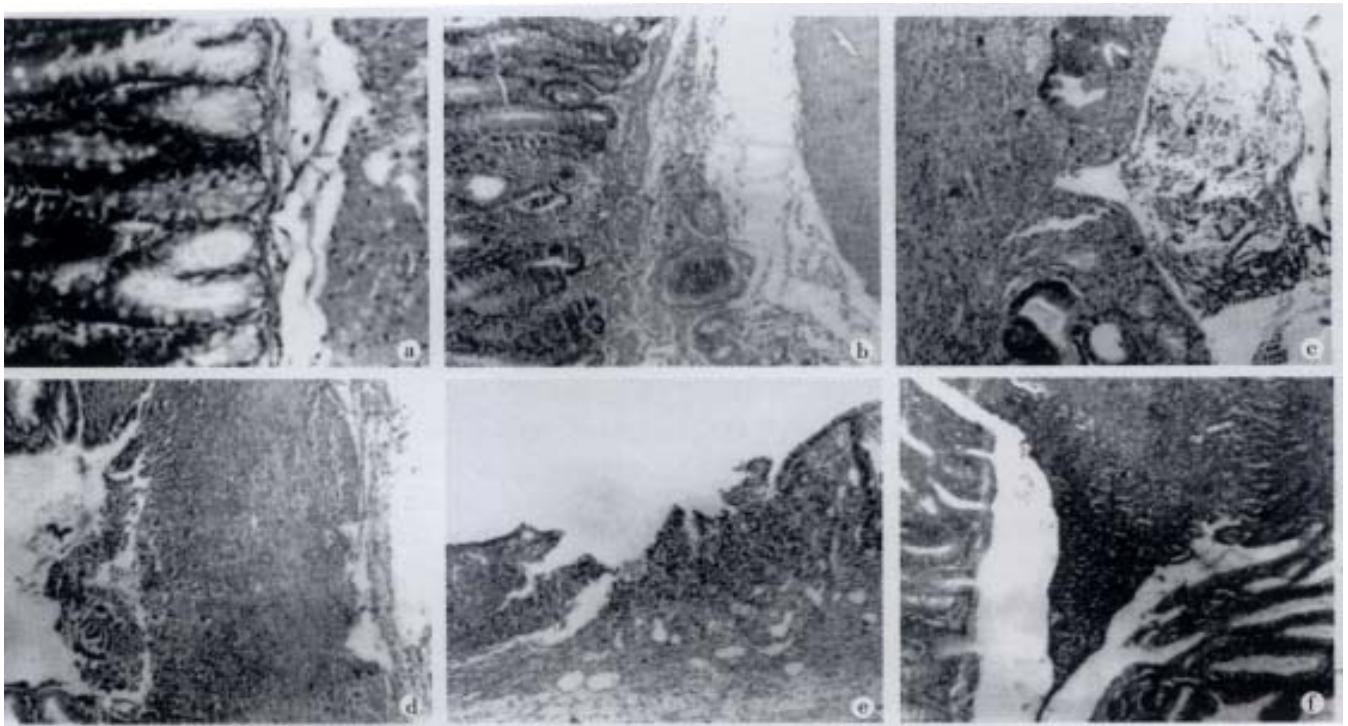


Figure 2 Histological findings in ulcerative colitis induced by different doses of TNBS ethanol. a: TNBS 25 mg/kg, the mucosa is normal. b: TNBS 50 mg/kg, small mucosal ulcers and crypt abscess formation. c-d: TNBS 100 mg/kg, mucosal ulcers, inflammatory exudate, proliferous granulomas and cells infiltration. e-f: TNBS 150 mg/kg, gross ulcers and proliferous granulomas, necrosis of epithelium at mucosal surface and inflammatory cell infiltration (He, ×100).

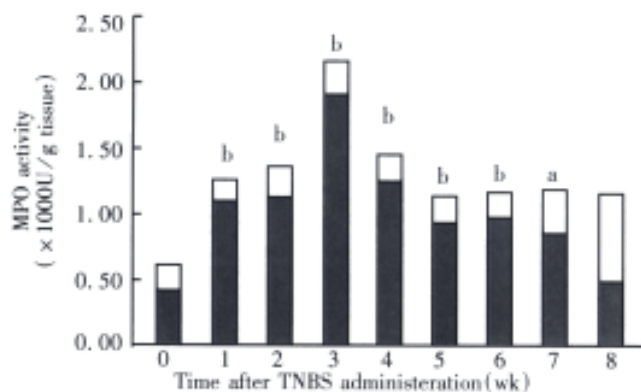


Figure 3 The effects of intracolonic administration of 100mg/kg of TNBS on MPO activity 1 wk-8 wk after administration. Each bar represents the $\bar{x} \pm s$ of 5 animals. All data for TNBS-treated rats were compared with 0 wk (control group). ^a*P*<0.05, ^b*P*<0.01.

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